B1 cont.

protein makes most contacts is designated the target strand, some zinc finger proteins bind to a three base triplet in the target strand and a fourth base on the nontarget strand. The fourth base is complementary to the base immediately 3' of the three base subsite.--

Please replace the paragraph beginning on line 19 of page 2 with the following:

B2

--A number of papers have reported attempts to produce ZFPs to modulate particular target sites. For example, Choo et al., *Nature* 372, 645 (1994), report an attempt to design a ZFP that would repress expression of a brc-abl oncogene. The target segment to which the ZFPs would bind was a nine base sequence 5' GCA GAA GCC3' chosen to overlap the junction created by a specific oncogenic translocation fusing the genes encoding brc and abl. The intention was that a ZFP specific to this target site would bind to the oncogene without binding to abl or brc component genes. The authors used phage display to screen a mini-library of variant ZFPs for binding to this target segment. A variant ZFP thus isolated was then reported to repress expression of a stably transfected brc-able construct in a cell line.

Please replace the paragraph beginning on line 17 of page 3 with the following:

B3

--The present application is related to copending applications 09/229,007 filed January 12, 1999 (WO 00/42219), now U.S. Patent No. 6,453,242 and 09/229,037 filed January 12, 1999 (WO 00/41566), and both incorporated by reference in their entirety for all purposes.--

Please replace the paragraph beginning on line 33 of page 3 with the following:

B4

--The invention further provides methods of selecting a dimerizing peptide. Such methods entail providing a phage display library in which a member displays a zinc finger protein fused to a peptide from its outersurface, the zinc finger protein being the same in different members, and the peptide varying between different members. The library is then contacted with a nucleic acid substrate comprising first and second binding sites for the zinc finger protein. Phage displaying a zinc finger protein fused to a dimerizing peptide preferentially bind to the substrate relative to phage displaying a zinc fusion protein fused to a nondimerizing peptide. The phage that bind to the substrate are isolated. A segment of the genome of a phage binding to the substrate is sequenced to determine the identity of a dimerizing peptide. In some such methods, the first and second binding sites are in opposing orientations in the substrate. In some methods, the phage displaying a zinc finger protein fused to a dimerizing peptide bind to

B4 cont.

the substrate via display of two copies of the zinc finger protein and the dimerizing peptide, whereby the two copies of the zinc finger protein respectively bind to the first and second binding sites, and the two copies of the dimerizing peptide bind to each other. In some methods, the peptide is a random peptide. In some methods, the peptide is 30 amino acids or fewer in length.--

Please replace the paragraph beginning on line 6 of page 10 with the following:

B5

--One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).--

Please replace the paragraph beginning on line 8 of page 11 with the following:

B6

--The application provides methods for selecting dimerization peptides that mediate association of linked functional proteins domains. The peptides can mediate such association by homodimerizing with each other, by heterodimerizing with the linked protein domains, or by binding to an entity, such as a DNA target site, itself bound by the linked protein domains. In particular, such peptides are useful for mediating association of complexes of multiple zinc finger proteins thereby affording greater specificity and/or affinity in binding of the zinc finger proteins to proximately spaced target segments.--

Please replace the paragraph beginning on line 18 of page 17 with the following:

B7

-- Zinc finger proteins are often expressed with a heterologous domain as fusion proteins. Common domains for addition to the ZFP include, e.g., transcription factor domains (activators, repressors, co-activators, co-repressors), silencers, oncogenes (e.g., myc, jun, fos, myb, max, mad, rel, ets, bcl, mos family members etc.); DNA repair enzymes and their associated factors and modifiers; DNA rearrangement enzymes and their associated factors and modifiers; chromatin associated proteins and their modifiers (e.g. kinases, acetylases and deacetylases); and DNA modifying enzymes (e.g., methyltransferases, topoisomerases, helicases, ligases, kinases, phosphatases, polymerases, endonucleases) and their associated factors and modifiers. A preferred domain for fusing with a ZFP when the ZFP is to be used for repressing expression of a target gene is a the KRAB repression domain from the human KOX-1 protein (Thiesen et al., *New Biologist* 2, 363-374 (1990); Margolin et al., *Proc. Natl. Acad. Sci. USA* 91, 4509-4513 (1994); Pengue et al., *Nucl. Acids Res.* 22:2908-2914 (1994); Witzgall et al., *Proc. Natl. Acad. Sci. USA* 91, 4514-4518 (1994). Preferred domains for achieving activation include the HSV VP16 activation domain (see, e.g., Hagmann et al., *J. Virol.* 71, 5952-5962 (1997)) nuclear hormone receptors (see, e.g., Torchia et al., *Curr. Opin. Cell. Biol.* 10:373-383 (1998)); the p65 subunit of nuclear factor kappa B (Bitko & Barik, *J. Virol.* 72:5610-5618 (1998)and Doyle & Hunt, *Neuroreport* 8:2937-2942 (1997)); Liu et al., *Cancer Gene Ther.* 5:3-28 (1998)), or artificial chimeric functional domains such as VP64 (Seifpal et al., *EMBO J.* 11, 4961-4968 (1992)).--

Please replace the paragraph beginning on line 26 of page 22 with the following:

--ZFP polypeptides, dimerizing peptides linked to the same, and nucleic acids encoding fusion proteins of ZFPs and dimerizing peptides can be made using routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel et al., eds., 1994)). In addition, nucleic acids less than about 100 B8 bases can be custom ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company, ExpressGen Inc., Operon Technologies Inc. (Alameda, CA). Similarly, peptides can be custom ordered from any of a variety of sources, such as PeptidoGenic (pkim@ccnet.com), HTI Bio-products, inc., BMA Biomedicals Ltd (U.K.), Bio.Synthesis, Inc.--

Please replace the paragraph beginning on line 27 of page 29 with the following:

-- **Phage Display Libraries.** Phagemid vectors used in the selections were created from pZif12 (7) by restoring the reading frame between the Zif12-coding region and gene III and by introducing convenient restriction sites at the start of Zif12. Libraries containing randomized peptides were constructed by cassette mutagenesis, using NN(G/C/T) randomized condons for B9 the initial libraries and NN(G/T) for the reoptimization libraries. The complete fusion protein used for phage display contained a PelB signal sequence; a short leader peptide (NH_2-EPRAQNS in initial selections and NH_2-EP in reoptimizations); the random peptide; residues 4-60 of Zif268 (numbering as in ref. 8); a linker that includes an amber condom; and residues 23-424 of M13 gene III product. The ligated phagemid libraries were electroporated into XL-1 Blue *E. coli* cells, yielding $\approx 10^8$ transformants for the initial selection libraries and $\approx 10^9$ transformants for each of the reoptimization libraries.--

Please replace the paragraph beginning on line 15 of page 31 with the following:

-- Labeled DNA probes were generated as follows. For the gel-shift studies, oligos corresponding to the phage-selection target site
B10 (5'GGTTGCAG**TGGGCGCGCCCA**CAGTACTTGAACGTAACG-3' and 5'-CGTTACGTTCAAGTACTG**TGGGCGCGCCCA**CTGC-3', Zif12 sites in bold) or a single-site mutant (bold regions above replaced with the sequences 5'-**TGGGCG**<u>TATGCT</u>-3' and

5'<u>AGCATA</u>**CGCCCA**-3') were annealed and end-labeled with Klenow. A labeled restriction fragment was used for quantitative studies. The oligos 5'-GGAATTCCTGA-TCAAGATCTGGTCACGTCCATAGGCTAGGCATGTCAAGGCTGTATG-3' and 5'-GGGATCCACTCGCGAACGCGTCCTTGTAG**TGGGCGCGCCCA**CATACAGCCTTGACAT-3' (Zif12 sites in bold) were annealed, extended by mutually primed extension, and cloned into the *Eco*RI and *Bam*HI sites of pBluescript II SK(+). The probe was prepared by digesting the plasmid with *Eco*RI and *Not*I; labeling the DNA with Klenow, (α-^{32}P)dCTP, and (α-^{32}P)dGTP; and purifying the small fragment by native PAGE.--

B10 cont

Please replace the paragraph beginning on line 26 of page 32 with the following:

--To select dimerization motifs, we attached random peptides to a DNA-binding domain and selected those fusion proteins that could bind more stably to a symmetric DNA site. Random 15- and 30-residue peptides were expressed at the amino terminus of the first two zinc fingers of Zif268 (8, 17) (we refer to this two-finger peptide as Zif12), and these peptide-Zif12 fusions were displayed on filamentous bacterio-phage. Phage from the 15- and 30-mer libraries, representing 10^8 different sequences from each library, were pooled, and our affinity-selection protocol was used with a target DNA duplex containing an inverted repeat of the Zif12-binding site. The original Zif12 peptide, which lacks any N-terminal extension, binds specifically, but weakly, to the "half-site" sequence TGGGCG, and Zif12 phage are not retained by the target DNA. Therefore, our protocol enriches for phage that display peptides that augment the DNA-binding activity of the zinc fingers.--

B11